Development of pseudo cereal quinoa based gluten free product to manage celiac disease among children

Abstract

Background and objectives: Celiac disease is an autoimmune enteropathy induced by gluten - protein found in various cereal grains.The present study was undertaken to develop quinoa flour based gluten free biscuits for its supplementation in celiac children (7 to 9 years). Gluten free biscuits were nutritionally analysed and effect of supplementation was studied by assessing nutritional statusof the subjects.

Findings: The nutrition analysis of most acceptable gluten free biscuits with 40 percent quinoa flour had appreciable amount of protein (11.45g), lysine (3.69g),tryptophan (0.58g), methionine (0.96 g),iron (1.77 mg), calcium (49.43 mg), magnesium (70.08 mg), zinc (1.77 mg) and fibre (2.45 g)per 100 g. A significant increase (p<0.01) in the intake of protein, iron and calcium was observed in children aftersupplementation.

Conclusion: Supplementation resulted in significant decrease (p<0.01) in Anti-tTG IgA levels from 116.88 to 57.19 U/ml andsignificant increase in haemoglobin levelfrom 11.59 g/dl to 12.1 g/dl. Besides, Z- Scores of normal BMI for age improved from 16.6 t to 57.2 %.

Significance and novelty: Quinoa based gluten free biscuits could be nutritionally superiorand healthy alternative which could prove a boon to manage celiac disease among children in developing countries.

Keywords: Quinoa flour, Celiac Disease, Supplementation, Anti-tTG IgA

Introduction

Celiac disease is an autoimmune enteropathy induced by gluten - a group of proteins found in various cereal grains. It is most common than is perceived in India as every 1 in 96 among north Indian community is suffering from celiac disease (Makharia et al., 2011). The prevalence of celiac disease and latent celiac disease is 8.53, 3.70 out of 1,000 in northern, 4.66, and 3.92/1000 in north-eastern and 0.11, 1.22/1000 in southern part of India. Dietary intake pattern of wheat is significantly highest in northern (455 g) followed by north-eastern and southern part of India (Ramakrishna et al., 2016). On the basis of epidemiology, occurrence of celiac disease (CD) is predominantly higher in wheat consuming states such as Punjab, Haryana, Delhi, Rajasthan, Uttar Pradesh, Bihar and Madhya Pradesh (Deora, Deswal, Dwivedi and Mishra, 2014). The prevalence of celiac disease was found to be significantly ($p<0.001$) higher in children than in adults (0.5 %) i.e. 0.9 % in children and 0.5 % in adults (Singh et al., 2018). Celiac disease prevalence amongst school going children in Punjab was observed to be one in 310 (Sood, Midha, Sood, Avasthi and Sehgal, 2006).Dietary and life style modificationsof the individualare proven effectivein reducing the risk of celiac disease. Improvement is mostly seen by including gluten free products in diet or complete withdrawal of gluten from diet (Penagini et al., 2013). Gluten free diets ameliorate the health of the celiac patient as it lessens the symptoms and normalizes the villi, which is of great importance for gastrointestinal functioning.Rising demands for gluten free products parallels the increase in celiac disease. Products made from corn, rice, soybean, tapioca, amaranth seeds and pseudo-cereal such as quinoa can be included in the diet. Quinoa is gluten free and can be used to prepare various gluten free products safe for celiac patients (Zevallos et al., 2014).

Quinoa (*ChenopodiumquinoaWilld*) is a dicotyledonous annual plant which belongs to the family *Amaranthaceae*, originated in the Andes region of Bolivia and Peru also known by different namei.equinua or simply quinoa. The United Nations General Assembly proclaimed 2013 as "The International Year of the Quinoa" in acknowledgment of tribal practices of the Andean individuals, who have protected quinoa as food for present and future generation through learning (United Nation, 2011). For the developing Indian population, quinoa seeds can be nutritious as compared to rice and wheat (Bhargava, Shukla and Ohri, 2006).

Quinoa is a treasure trove of nutrients. As compared to rice, corn, rye and sorghum, the protein content of quinoa (13.8-16.5%)is higher and is almost similar to that of wheat (USDA, 2015). It has balanced set of essential amino acids such as methionine, lysine and cysteine whereas wheat, rice and corn are low in lysine (Maradini et al., 2015). It has low concentration of prolamines. Quinoa is considered to be excellent source of antioxidant vitamin E and moreover B-complex vitamin levels are higher than the other grains (Alvarez, Arendt and Gallagher, 2010).Quinoa seeds contains appreciable amount of magnesium, zinc, iron, calcium and considered as sufficient for balanced diet. Quinoa contains a high level of protective phytochemicals such as saponins, phytosterols, phytoecdysteroids (Navruz and Sanlier, 2016).

Germination of quinoa grains can bring the desirable changes in the texture and sensory characteristics of the product and also improves the nutritive value of the product. Germination of quinoa showed a significant effect on the protein, ash, crude fibre, and starch and carbohydrate contents of white and red varieties. Also, Significant ($p \leq 0.05$) increase in antioxidant activity was observed with the significant increase in phenolics, flavonoids and ascorbic acid content (Padmashree et al., 2019).Therapeutic properties and gluten free nature of quinoa benefits high risk group consumers, including youngsters, elders, and individualswith lactose-intolerance, women prone to osteoporosis, diabetes, dyslipidaemia, obesity and celiac disease (Galvez et al., 2010). So, Quinoa can prove a boon for celiac patients.

Quinoa can be used to make novel, healthy, extruded and snack type food products. Quinoa flour can be used at different levels in product development such as bread, sweet biscuits (10-60%) and extruded products (30-40%) (Zevallos et al., 2014). Due to its endurance at low storage temperatures, low gelling point and good freeze-thaw stability, it can also be used as thickener in soups, sauces and flours. It can be used alone or in combination with other food material such rice flour, oat flour, bengal gram flour for development of various value added products such as cookies, biscuits, bread, salad and popup, extruded snacks up to 25% level. Quinoa can be considered as excellent novel source as natural health promoting food.

Choices of foods are limited for celiac patients and are not nutritionally balanced. Substitute for the wheat, having cereal base should be developed. As Quinoa is an emerging novel pseudo cereal with various health benefits and not much clinical trials have been done so far. Therefore, the presentstudy was conducted with the objectives to develop and conduct organoleptic evaluation of quinoa flour based gluten free biscuits, to determine the availability of nutrients from acceptable biscuits and to evaluate the effectiveness of quinoa to manage celiac disease among children.

Material and methods

Procurement of raw material

Quinoaseeds (white variety) were procured from local farmer of Ludhiana. The other materials such as sugar, butter, milk, rice flour and maize flour used to develop gluten free biscuits were purchased from local market of Ludhiana.

Processing of quinoa grains

Quinoa grains were thoroughly cleaned in running water to remove saponins. Quinoa grains were soaked overnight in water at room temperature and were kept for germination for a period of 24hours. The germinated grains of quinoa were dried at 50°C in the hot air oven for 6hours. After drying, the quinoa grains were milled into fine flour using dry milling process in the Department of Food Science and Technology, PAU, Ludhiana. Developed flour was stored in air tight containers for further use.

Development of gluten free biscuits

Experimental samples of gluten free biscuits were developed with the combination of rice and maize flour and by incorporating different levels of quinoa flour(10-60%) while the control sample was prepared from 80 % rice and 20 % maize flour. Gluten free biscuits were prepared in the Experiential Learning Unit of Department of Food and Nutrition, College of Community Science, PAU, Ludhiana, India.Biscuits were developed using standardized recipe (Kochhar and Kaur, 2015). For the preparation of gluten free biscuits, different proportions of gluten free flours were sifted and blended thoroughly along with baking soda (2 g) and baking powder (2 g). For creaming purpose,55 g of butter and 50 g of powdered sugar were rubbed together on the clean surface. Blended flour was added to the creamed mixture and milk (10 ml) was used to obtain the smooth dough. The sheet of about 0.5cm of thickness was obtained from the dough and round shapes were carved using mould. Biscuits were bakedin deck ovenat 150°C for 20 minutes. The biscuits were cooled for half an hour at room temperature and stored in low density polyethylene packs for further analysis.

Organoleptic evaluation

Ten semi- trained panellists carried out sensory evaluation of developed biscuits. Two sessions of one hour each were conducted to train panellists so as to familiarize themselves with the samples. In first session, the panel was served with 80 % rice and 20 % maize flour supplemented gluten free biscuits along with experimental samples prepared using 20, 40 and 60 percentquinoa flour in different ratios viz 20:60:20, 40:40:20 and 60:20:20 (quinoa: rice:

maize). During second session, the panellists were able to understand the test and were given a score sheet to evaluate sensory attributes, i.e.colour, taste, texture, flavour and overall acceptability.Thepanellists were asked toscore samples on nine point hedonic rating scale where scores 1, 2, 3, 4, 5, 6, 7, 8 and 9 illustrated dislike extremely, dislike very much, dislike moderately, dislike slightly, neither like nor dislike, like slightly, like moderately, like very much and like extremely, respectively (Rangana, 1986).All panelists were instructed to rinse their palate with mineral water before testing each sample. The product characterization was carried out under "day light" illumination and in isolated booths within nutrition laboratory.Samples was given different codes to avoid any biased judgement and mean score for each sample was calculated. Experimental sample with 40 % quinoa flour got the highest scores of all sensory attributes amongst control as well as other experimental samples.

Nutritional and anti-nutritional analysis

Biscuits prepared by using 80 % rice flour and 20 % maize flour along with experimental sample supplemented with 40 % quinoa to 40% rice and 20% maize flour were weighed and uniformly grounded and dried at 60°C in oven. Dried ground samples were stored in air tight containers for further nutritional analysis.The proximate composition (AOAC, 2000), amino acids –tryptophan (Concon, 1975); available lysine (Booth, 1971); methionine (Horn, Jones and Blum, 1946), minerals (Piper, 1950) – calcium; magnesium; iron; zinc and anti-nutritional factors – saponins (Domengza, Steinbronn, Francis, Focken and Becker, 2009); phytin phosphorus (Haug and Lantzsch, 1983)were determinedusing the standard methods.

Supplementation trial

A group of sixty celiac children in the age group of 7 to 9 years age group regardless of gender were taken from local hospitals of Ludhiana, Punjab, India.A questionnaire was developed to obtain information pertaining to demographic, socio-economic status, dietary pattern, medical history,food intake, anthropometric measurements and biochemical parameters. Pre-testing of the questionnaire was carried out on 10 young children to check its validity and suitability. For supplementation trial,celiac children were divided into two groups namely experimental and control having 30 each. The celiac children of experimental group were provided six (50±5 g) developed biscuits using quinoa, rice and maize(40:40:20) per day for a period of three months.The same amount of rice and maize biscuits (80:20) were given to celiac children in control group.

Data collection

The effect of supplementation was determined by taking data pertaining to nutritional status of celiac children such as dietary intake, anthropometry and biochemical parameters. The data was collected at two time intervals before and after supplementation for three months. The supplementary effect was assessed by observing increase and decrease in all the parameters used to assess nutritional status of young children.

Dietary intake: Dietary intake of subjects was recorded for three consecutive days using 24 hour dietary recall method using standardized measuring containers.Data pertaining to nutrientintake was calculated using 'Diet Cal software' (Kaur, 2014).Mean daily food intake and nutrient intake was compared with suggested dietary intake (SDI) and estimated average requirement (EAR) as given by Indian Council of Medical Research (ICMR, 2010; ICMR, 2020).

Anthropometric measurements: Height was measured by using flexible, non-stretchable fibre glass tape. The subjects were told to stand barefoot along the wall and erect the head comfortably. Head piece was lowered, touching the top of the head with scale and height was measured by marking on wall with the pencil. For weighing the subjects, weighing balance was used. Subjects were told to remove shoes and stand straight at the centre of the platform. Weight was recorded in Kilograms (Kg), up to 0.2 Kg accuracy. For the measurement of mid upper-arm circumference (MUAC), fibre tape was placed firmly around the left upper-arm, mid-way between acromial and olecranon according to the compression of soft tissues and the measurements were noted down.The Z-score for three indices visualizing weight-for-age (WAZ), height-for-age (HAZ) and BMI-for-age were computed by using ‘WHO: Anthro-plus’ software (WHO, 2009). The prevalence of malnutrition was calculated on the basis of Z-score cut off level:>+2.0 SD: Overweight;-2.0 to +2.0 SD: Normal; <-2.0 SD: Moderately malnourished; < -3.0 SD: Severely malnourished (WHO, 2006).

Biochemical parameters: The biochemical parameters mainly Haemoglobin (Dacie and Lewis, 1989), Packed Cell Volume (Raghuramulu, Mahadevan and Kalyana, 1983), Mean Corpuscular Volume, Red Blood Cell count (Hunter and Bomford, 1967) and Anti-tissue Transglutaminase Immunoglobulin A(NHANES, 2009-10)were assessed using standard methods.

Statistical analysis

Data were analysed using appropriate statistical tools such as mean and standard deviation. Kruskal-Wallis and Mann-Whitney test was applied using Statistical Package for the Social Sciences (SPSS-16.0) to select the best formulation of developed gluten free biscuits. Student *t*-test was applied to test the significant difference in nutrient compositionof control and experimental sample of gluten free biscuits. Paired *t*-test was used to analyse the data pertaining to dietary intake, anthropometric and biochemical parameters of the celiac children.

Results And Discussions

Quinoa is highly nutritious due to its outstanding protein quality, mineral content and have a good source of antioxidants, bioactive compounds as compared to other cereals and pseudo – cereals (Debski, Mikolaj, Gralak, Bertrandt and Klos, 2013; Hirose, Fujita, Ishi and Ueno, 2010).Quinoa flour based gluten free biscuits were developed as healthy alternative for celiac children. So, it was obligatory to perform acceptability trials. Quinoa flour based gluten free biscuits developed by using combination of rice and maize flour by incorporating 40 percent of quinoa flour got the highest scores in sensory attributes. Before starting the supplementation trial, the approval was taken from PAU ethical committee(No. DR-8271-80-19-9-19), Ludhiana, Punjab. The permission for supplementation was also obtained from the parents of selected celiac children.

Nutritional evaluation

Quinoa seeds possess 10.74-11.77 % of moisture, 3.22-3.87 % of ash, 56.69-66.07 % carbohydrate, 11.15-17.81 % crude protein, 4.01-6.14 % crude fat and 6.30-8.24 % crude fibre (Barakat, Khalifa, Ghazal, Shams and Denev, 2017). In present study,most acceptable gluten free biscuitssupplemented with 40 percentquinoawere chemically analysed for their nutritional as well as anti-nutritional composition (Table I). The results revealed that crudeprotein content of the quinoa based biscuits was significantly ($p \leq 0.05$) higher(11.45%) thancrude protein of the control sample (8.99%).Protein content was found to be significantly higher in sweet quinoa varieties i.e 14.9 and 15.8 % in bitter quinoa(Wright, Pike, Fairbanks and Huber, 2002). Crude fibre content of quinoa based biscuits was reported to be 2.45 % ,whichwas significantly higher ($p \leq 0.01$) than that of control sample(0.94%) .Theoutcomesofcurrent study coincide with the findings of previous researchers who reported that quinoa flour is good source of protein (12.9-16.5%) and dietary fibre comprising about 2.6 to 10 percent of the total weight of the grain (Gordillo, Rizzolo, Roura, Massanes and Gomis, 2016; Lamothe, Srichuwong, Reuhs and Hamaker, 2015).The total dietary fibre and soluble fibre content of quinoa seeds was reported to be 7-11.7 percent and 1.3 – 6.1 percent (Gebhardt et al., 2008).The biscuits blended with 40 percentquinoa flour showed the highest protein and crude fibre content of 25.37 and 1.16 %, respectively (Puri, Sarao, Kaur and Talwar, 2020).

Quinoa flour contains appreciable amounts of minerals. The mineral content such as calcium, magnesium and potassium were found in sufficient quantities for a balanced diet ranging from 874mg/kg, 1901.5 mg/kg and 9562.2 mg/kg(Gordillo, Rizzolo, Roura, Massanes and Gomis, 2016).Results of present study revealed that quinoa based biscuits hadsignificantly higher ($p \leq 0.01$) iron content of 1.77 mg/100g than the control sample having 1.06 mg/100g. The calcium and magnesium content in control sample was 10.50 mg/100g and 22.49 mg/100g which significantly ($p \leq 0.01$) increased to 49.43 mg/100g and 70.08 mg/100g after addition of 40 % quinoa flour in gluten free biscuits.The results are in line with the findings ofSohaimy, Mohamed, Shehata, Mehany and Zaitoun (2018) that quinoa flour had the most balanced content of essential minerals as compared with the other cereal grains. Iron content in quinoa flour was 6.5 times the iron content of wheat which emphasizes the benefit of quinoa flour for malnutrition. Quinoa flour possess high levels of minerals such as iron (63 mg/ Kg), magnesium (174 mg/ Kg) ,potassium(443mg/Kg),sodium (858 mg/ Kg) and calcium (127 mg/ Kg) .Zinc content of 1.51 mg/100g in quinoa based biscuits was significantly higher ($p \leq 0.01$) than the control sample with zinc content of 0.37 mg/100g. Similarly,another studyreported that quinoais a potential source of minerals as it has a good content zinc (3.65 mg/100 g), iron (5.40 mg/100 g) and calcium (176 mg/100 g) which was higher than that of the present findings (Lazarte, Carlsson, Almgren, Sandberg and Granfeldt, 2015).

The dietary protein availability and protein metabolism are dependent upon the protein digestibility and amino acid composition present in the food. The analysis with regard to amino acid composition of the biscuits revealed a significant ($p \leq 0.05$) difference in lysine content of quinoa flour based and control biscuits (Table I). quinoa based biscuits had significantly higher lysine content than the control sample (3.69 vs 2.28g/100g protein).Similar trend was observed in previous study conducted by Elsohaimy, Refaaya and Zaytounb (2015) that quinoa protein possess highest level of lysine (17.13g/100g) and moderate levels of methionine (1.70g/100g).

The estimated values of tryptophan and methionine in quinoa based biscuits were 0.58 and 0.96 g per 100 g protein which was significantly higher ($p \leq 0.01$) than that of control sample with tryptophan and methionine content of 0.43 and 0.54 g per 100g protein, respectively.Overall, the amino acid content was found to be higher in biscuits prepared from quinoa flour as compared to control.Quinoa seeds contains appreciable amount of protein and incredible balance amino acids (Navruz and Sanlier, 2016).

The anti-nutritional analysis revealed that the saponin content was found only in the experimental sample supplemented with 40% quinoa flour i.e. 0.48%. The present results are corroborated with the findings of Lim, Park and Yoon (2020) who found that quinoa seeds possess 1.26g/100g of saponin content which was reported to be significantly higher than the saponin content of quinoa stem, quinoa leaves, and quinoa roots. Antinutritional factors such as phytates, oxalates, tannins, trypsin inhibitors and saponins were found higher in outer layers of cereals. Saponin content and bitterness can be easily removed by wet and dry methods(Maradini et al., 2015) .Germination of quinoa results in significant reduction in antinutritional factors (Ananthan et al., 2019). One of the study documented that quinoa contained 557 mg/ 100 g saponin content and it was reduced to 36 mg/ 100g after abrasive milling (Pirjo et al., 2018).Phytin phosphorous content in the quinoa based biscuits (38.45 mg/100g) was significantly ($p \leq 0.05$) higheras compared to the control sample (19.03 mg/100g).Similarly, another study also reported higher concentration of phytates in quinoa i.e. 2060 mg/100g (Lazarte, Carlsson, Almgren, Sandberg and Granfeldt, 2015).

Demographic profile and socio-economic status

The demographic profile of celiac children is given in Table II. Majority of the children (60 %) were between 8 to 9 years of age and 40% were of 7 to 8 years age group. A major proportion of children (63.33 %) were males while 36.67 percent of children were females. In contradiction, Zingoneet al. (2015)documented that among 1247 children, celiac disease incidence was higher in girls than in boys.Majority of the subjects i.e. 53.33% were of the first birth order, followed by second birth order (43.33 %) and third birth order (3.33 %). Birth order might affect the nutritional status of children and the possible reason could be that the children with increasing birth order are more likely to be unwanted which may results in less attention and care from parents. Child check-up also decreases with the higher birth order. As a result, children with higher order of births might suffer from various health hazards as well as malnutrition (Khan and Raza, 2014).The data related to family type highlighted that most of the subjects (76.67 %) belonged tonuclearfamilieswhile23.33% of the subjectswere fromjoint families.

The information regarding socio-economic status revealed that monthly family income of thirty percent children was between Rs.25,000-50,000 i.e. low, 36.67 percent children families have medium income (Rs.50,000-75,000), 13.33 percent families had high income (Rs.75,000-1,00,000) and twenty percent families had very high income (above Rs.1,00,000). Roy et al. (2016) stated that patients with non-classic symptoms of celiac disease are less likely to be diagnosed when they belong to a lower socioeconomic status. Factors such as sex, age and low per capita income were considered a significant predictor for the diagnosis of celiac disease without any gastrointestinal symptoms.

As far as the education of children's parents was concerned, the results revealed that both the parents of selected children were well educated asmajor proportion of fathers (63.33 %) and mothers (60 %) of the subjects weregraduatesand none was illiterate. Igbokwe (2014) reported that factors such as age and sex, parental education and socioeconomic class had a significant impact on nutritional status. Children of parents with secondary education and below were more stunted, wasted and underweight than those whose parents had tertiary education. Children from the upper socioeconomic class were more overweight and obese while those from lower socioeconomic class were more stunted, wasted and underweight.

The data related to parents occupation indicated that most of the subject's father were working in a private firm (46.67 %) followed by 26.67 % having their own business,16.67 per working in government sector and 10% farmers. Major proportion of children's mothers (56.67 %) were having working status.Haines et al.(2019)stated that busy parents struggling to balance work and family life have less time to shop and prepare traditional food. Increase in parental stress is linked with consumption of less healthy foods and children are more likely to be overweight or have unhealthy eating habits.

Medical history

The data regarding the time of diagnosis of celiac disease as shown in Table III revealed that 46.67% of the selected children had been diagnosed for more than twelve months, followed by 30 % diagnosed for less than six months and 23.33 % for less than six months. Almost 40 % of the subjects were having family history of the disease. The reason behind the family history of celiac disease might be that it is a genetic hereditary disease.Celiac disease was found to be more prevalent among first degree relatives of the subject i.e. mother (58.33 %) and father (50 %). Singh, Arora, Lal, Strand and Makharia (2015) observed that among 10,252 first degree relatives and 642 second degree relatives of the celiac patient, the prevalence of celiac disease was found to be 7.5 and 2.3 percent, respectively.Prevalence of celiac disease was reported to be higher among female first degree relatives than male first degree relatives.Likewise, another study reported that the prevalence of celiac disease varied from 4.5 percent among first degree relatives of celiac patient to 0.5-1.0 % in general population (Fasano et al., 2003).

Morbidity record of the celiac subjectsindicated that among sixty celiac children, 63.33 percent of subjects were holding sickness record for past one year. The highest sickness observed was fever (73.68%), followed by cough and cold (68.42%) and viral infection (21.05%).Almost 4.17 % of subjects with morbidity record of only experimental group suffered from chicken pox and dengue. The possible reason behind these 63.33 per cent of the subjects with morbidity could be the malabsorption caused due to celiac disease which tends to make celiac patients more susceptible to illness than their otherwise healthy counterparts. Moreover, the delay in the diagnosis of celiac disease is related with the poor health and reduced well-being and also linked with the increased use of medicines and health care services (Fuchs et al., 2018).

The information related to signs and symptoms revealed that abdominal pain was observed in seventy percent of the subjects. Nearly half (53.33%) of the total selected celiac subjects were reported with breathlessness. Common complaints such as dyspnea which includes asthma, myocardial dysfunction, chronic obstructive pulmonary disease and pulmonary embolus were reported in celiac disease patients (Kanwal, Sheikh, Biyyani, Sievers and Kyprianou, 2012). Fatigue was prominent among sixty percent of the subjects. Irregular heartbeat, breathlessness, fatigue, flat nails and paleness might be caused due to iron deficiency anemia as iron deficiency is a very common feature of celiac disease. Blistering skin rashes were observed in 63.33 percentofthe subjects.Salmiet al.(2015) reported that dermatitis herpetiformis is most often an indicator of poor adherence to gluten free diet andit may develop by time in celiac patients. The subjects with chronic diarrhoea and foul smelling stools were 46.67 and 76.67 % respectively. Nearly forty percent of the subjects were having canker sores inside their mouth. Almost 43.33 percent of the subjects faced hardships in memorizing.

Supplementary effect of biscuits on nutritional status of celiac children

The effect of supplementation of gluten free quinoa based biscuits on nutritional status of celiac children was expressed in terms of change in food and nutrient intake, anthropometric measurements and biochemical parameters. The prevalence of malnutrition, Anaemia and Anti-tTG IgA levels were also recorded (Figure I).

Dietary Pattern

The data related to type of food consumed by the celiac children in Table Vrevealed that the subjects of both control and experimental group consumed homemade as well as readymade food before the Supplementation. The parents of both groups were very much aware about the availability of gluten free products available in the market.After supplementation trail, the number of experimental subjects consuming readymade food was observed to be decreased. Penaginiet al.(2013) reported that limited choice of food products in the diet of celiac children results in high consumption of packaged gluten free products such as snacks and biscuits. Patron and Barca (2017) stated that traditional homemade meals got replaced byultra- processed foods and ready to eat products because of their ease and availability, but they bring on negative impact on health due totheir low-fiber and high-fat and sugar composition. Genetically predisposed children should avoid ultra-processed food products so as to decrease the susceptibility of celiac disease.All the subjects of experimental group consumed quinoa based gluten free biscuits during supplementation period while the subjects of control group consumed gluten free biscuits without quinoa.Valituttiet al. (2017) observed significant differences in the consumption of bread, biscuits, and crackers by the celiac patients and control population. The consumption of biscuit and cracker was found higher (165.8g vs 22.7g and 44.7g vs 10.6g, $p<0.05$) among children in experimental group than in control.

Food and nutrient intake

The data regarding the food intake by the subjects of both groups in Table VIreported an inadequate food intake of all food groups except sugars, fats and eggs before supplementation. After supplementation trail, a significant ($p\leq0.01$) increase in the intake of cereals and millets, roots and tubers, fruits, milk and milk products, pulses and legumes($p\leq0.05$) and eggs ($p\leq0.05$) by experimental group subjects was observed. However, the control group showed non-significant improvement in all the foods except other vegetables, animal meat, fish and eggs.Similarly, a significant ($p \leq 0.01$) increase in the intake of nutrients such as energy, protein, carbohydrates, iron, calcium and thiamine ($p \leq 0.05$) was observed in the experimental subjects after the supplementation whereas non-significant improvement in the nutrient intake except iron, folic acid and vitamin C was observed in the control subjects.After supplementation, mean daily dietary intake of protein significantly($p \leq 0.01$) increased by 13.72 percent from26.32 g to 29.93 g in the experimental and non-significant increase by 7.5 percent in the control subjects i.e.from 24.66 to 26.51 g was observed. The percent adequacy of protein intake also increased from 129.78 to 139.52 percent after supplementation in the experimental group. However the protein intake was found higher than the EAR (19 g/day) in both the groups before and after the supplementation. Zuccotti et al. (2013) supported the present results by reporting that the dietary protein intake was significantly higher among the celiac children and exceeded the National recommendations for health and also, median energy intake was observed to be significantly ($p < 0.001$) higher in celiac patients than in controls.

A significant increase ($p \leq 0.01$) by 30.42 percent was observed in the mean daily iron intake of the experimental subjects as it increased from 10.06±2.20 mg to 13.12±1.82 mg after the supplementation whereas non-significantly decrease by 5.06 percent from 12.26±1.96 mg to 11.64±2.17mg was observed in the control subjects.The percent adequacy of ironincreased from 100.6 to 131.2 % in experimental groupafter supplementation.Likewise, Ohlund, Olsson, Hernell and Ohlund (2010) found that the nutrient density of iron was higher in celiac children than in controls.

Anthropometric profile

The anthropometric data of the celiac children revealed that the average height of selected subjects increased by 0.15 %in the control group while the corresponding figure in experimental group was 0.08 % (Table VII). Further it was observed that average height of control and experimental children was 127.22±7.49 and 127.02±9.01 cm, respectively which non-significantly increased to 127.42±7.68 and 127.12±9.00 cm after the supplementation of gluten free biscuits. Soliman et al (2019) reported that among 26 celiac children, the follow up of gluten free diet resulted in fast-linear growth (increased Ht-SDS) in seven of the man catch-up growth occurred in some of them after 2 years of being on gluten free diet. In contrast, one previous study reported that there was significant statistical difference ($p<0.01$) in the mean height of gluten free diet compliant children (130.47 cm) as compared to the non-compliant children (112.19 cm) (Khurana et al., 2015).

In terms of weight of the selected celiac children, the average weight of celiac children in both the groups remained lower than the standard value (25.3 kg) given for children in age group of 7-9 years (ICMR 2020). A significant ($p\leq0.01$) increase and maximum percent increase in weight was observed in experimental group (6.86 %) after the supplementation whereas non-significant increase was observed in control group from 20.84±3.03 to 21.02±2.87 kg with percent increase of 0.86%. The results of present investigation are in collaboration with the findings of Zuccotti *et al* (2013) who revealed that gluten free products consumption accounted for 36.3 % of daily total energy intake and the median energy intake was observed to be significantly ($p < 0.001$) higher in celiac patients than in control.Weight gain was reported in the celiac patients who strictly adhered to the gluten free diet. Nearly 15.8 % of patients with celiac disease had a move from a normal or low BMI class at diagnosis to an overweight BMI classand 22 percent of those already overweight at diagnosis gained more weight (Kabbani et al., 2012).

Mid upper arm circumference (MUAC) of experimental subjects was 19.84 cm, which significantly ($p\leq0.05$) increased to 19.90 cm after the supplementation period whereas non-significant improvement was observed in MUAC of control subjects. Likewise, significant increase ($p\leq0.05$) in the body mass index of about 6.70 % was observed in the experimental group after the supplementation. The significant increase of BMI might be due to the increase in weight of the subjects after the supplementation. For instance, one study revealed that that celiac children were found significantly (p= 0.014) less overweight or obese than healthy controls i.e 12 and 23.3 % respectively and significantly (p<0.001) more frequently underweight than healthy controls i.e 16 and 4.5 %, respectively (Brambilla et al., 2013).

Prevalence of malnutrition

Perusal of the data related to prevalence of malnutrition in celiac children is presented in Figure I. Low height-for-age refers to stunted growth, can be used as a standard, which assesses chronic malnutrition. Z-score for the height-for-age revealed that stunting was present in the subjects of both groups before and after supplementation periods. Moreover, the moderate type of stunting was noticed to be more among experimental group (33.33 %) than the control group (26.7 %). Bhavika et al. (2020) found that stunting was present in 61.1 % of celiac children and 5.6 % of the control children. Nearly 50 % of celiac children and 2.8 % of normal children had wasting. Igbokwe (2014) also reported that the prevalence of stunting, underweight and wasting among the primary school children were 0.8 , 3.3 and 3 % respectively, while the prevalence of overweight and obesity were 8.5 and 4.1 % respectively.

Weight-for-age is an index of the adequacy of the child's nutrition to support growth, which is a relevant marker for underweight, corresponds to the extended malnutrition. The large number of subjects of both the groups were found to be underweight before supplementation. The supplementation resulted in great increase in the number of normal weight subjects of experimental group from 46.7 to 73.3 % while the number of normal weight control subjects increased from 40 to 53.4 %. Similar trend was observed in the Z-score for BMI-for-age of the subjects. Supplementation showed a great effect on the Z-score of BMI-for-age of the experimental subjects as the scores of normal BMI-for-age improved from 16.6 to

57.2 %. Reilly et al. (2011) observed that nearly 75 % celiac children were having increased BMI at the time of diagnosis which significantly decreased to 44 %. The celiac children could manage to reduce their BMI Z-scores after strict follow up of gluten free diet. Weight Z-scores of celiac children with a normal BMI at the time of diagnosis, increased significantly after the follow up and 13 % of them became overweight.Wiech et al. (2018) also reported that gluten free diet resulted in a significantly ($p< 0.05$) higher increase in weight and body mass index in celiac children.

Biochemical parameters

The data regarding biochemical parameters of the celiac subjects is presented in Table VIII. It was noticed that subjects of experimental group were found anaemic before the supplementation as the mean haemoglobin level was 11.59 g/dl which was less than the normal range (12-17 g/dl). After supplementation, it was significantly ($p\leq 0.01$) increased to 12.1 g/dl with percent increase of 4.35 % whereas the haemoglobin of the control subjects non-significantly increased by only 0.55 % from 12.04 to 12.11g/dl. Narang et al. (2018) revealed that celiac disease was indirectly linked with moderate-to-severe anemia in 4% of the children.

Supplementation of quinoa based gluten free biscuits for the period of three months to the experimental subjects resulted in a significant decrease ($p\leq 0.01$) of anti-tTg IgA level from 116.88 to 57.19 U/ml. The levels declined by 51.07 % in experimental subjects whereas the anti-tTg IgA level in control subjects non-significantly decreased by 23.13% from 93.61 to 71.96 U/ml. However, even after supplementation, the anti-tTg IgA level of both the groups was reported to be higher than the standard value (< 7 U/ml).Upon analyzing the PCV, RBC count and MCV of the subjects, the data showed non-significant improvement in the subjects of both the groups after the supplementation.Kaur, Chinna, Bhat and Verma (2012) studied the prevalence of celiac diseases in symptomatic children in

Punjab. Anti tTG-IgA was estimated by ELISA and the results showed that 18 % children were having significantly higher levels of anti tTG-IgA (>20U/ml) in sero positive cases than sero negative cases i.e. 195.48 and 4.02 %. Sero positive cases were found to be higher in the age group of 6-10 years. Toma et al.(2019) reported that the reduction or total elimination of dietary gluten leads to a decrease in the levels of antibodies directed against gliadin or tTG. After 6–12 months of adhering to a gluten free diet, 80 percent of subjects will test negative by serology. Oliveira, Mohan and Fagbemi (2018) reported that 23 % of them were found anaemic, 63 % were having low ferritin and 62 % were observed with a moderate to severe deficiency of 25-hydroxyvitamin D. Twelve months follow up of gluten-free diet resulted in complete clinical improvement in 51% and partial in 49%. IgA tTG levels were found to be normalized in 45 percent of the selected celiac children after the follow up of gluten-free diet for 12 to 30 months.

Conclusion

The present research work explored that gluten free biscuits supplemented with 40 percent quinoa flour were nutritionally superior than maize and rice flour based biscuits. Supplementation resulted in significant decrease in anti-tTG IgA levels and also, improved percent adequacy of food and nutrient intake of celiac subjects. Supplementation was found to be effective tool in reducing the incidence of malnutrition and anemia among the celiac children. Therefore, the findings of current investigation suggested that quinoa flour in combination with maize and rice flour should be utilized to develop gluten free highly nutritious foods and highly recommended for long duration supplementation to celiac children for better result.

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Table I:Nutrient composition of quinoa based gluten free biscuits

Parameters	Control	Experimental	*t* value
Crude protein (g %)	8.99±0.45	11.45±0.74	4.009*
Crude fibre (g %)	0.94±0.03	2.45±0.04	35.10**
Energy (Kcal/100g)	613.86±3.45	634.44±4.44	5.17*
Minerals (mg/100g)			
Calcium	10.50±0.61	49.43±1.25	39.47**
Iron	1.06±0.11	1.77±0.07	7.36**
Magnesium	22.49±0.43	70.08±0.82	72.36**
Zinc	0.37±0.02	1.51±0.01	57.44**
Amino acids (g/100g protein)			
Tryptophan	0.43±0.01	0.58±0.01	13.58**
Methionine	0.54±0.01	0.96±0.02	21.16**
Lysine	2.28±0.29	3.69±0.18	5.70*
Anti-nutritional factors			
Phytin phosphorus (mg/100g)	19.03±3.75	38.45±2.39	6.16*
Saponins (%)	0.00	0.48	-

Biscuits: Experimental - quinoa, rice & maize (40:40:20)
Control - rice & maize (80:20)

Values are mean ± *SD*. Results are on dry matter basis. QF- Quinoa flour.
*Significant at 5 percent level of significance
**Significant at 1 percent level of significance

Table II: Demographic profile and socio-economic status of celiac children

Variables	Control (*n*=30)	Experimental (*n*=30)	Total (*n*=60)
Age (years)			
7-8	14 (46.67)	10 (33.33)	24 (40.00)
8-9	16 (53.33)	20 (66.67)	36 (60.00)
Gender			
Male	22 (73.33)	16 (53.33)	38 (63.33)
Female	8 (26.67)	14 (46.67)	22 (36.67)
Birth Order			
Ist	14 (46.67)	18 (60.00)	32 (53.33)
IInd	16 (53.33)	10 (33.33)	26 (43.33)
IIIrd	-	2 (6.67)	2 (3.33)
Type of Family			
Nuclear	24 (80.00)	22 (73.33)	46 (76.67)
Joint	6 (20.00)	8 (26.67)	14 (23.33)
Father's Education			
Senior Secondary	6 (20.00)	4 (13.33)	10 (16.67)
Graduate	20 (66.67)	18 (60.00)	38 (63.33)
Postgraduate	4 (13.33)	8 (26.67)	12 (20.00)
Mother's Education			
Senior Secondary	12 (40.00)	4 (13.33)	16 (26.67)
Graduate	16 (53.33)	20 (66.67)	36 (60.00)
Postgraduate	2 (6.67)	6 (20.00)	8 (13.33)
Family Income (Rupees/month)			
25000-50000	12 (40.00)	6 (20.00)	18 (30.00)
50000-75000	8 (26.67)	14 (46.67)	22 (36.67)
75000-100000	6 (20.00)	2 (6.67)	8 (13.33)
100000 or more	4 (13.33)	8 (26.67)	12 (20.00)
Father'sOccupation			
Private job	16 (53.33)	12 (40.00)	28 (46.67)
Government	6 (20.00)	4 (13.33)	10 (16.67)
Farmer	2 (6.67)	4 (13.33)	6 (10.00)
Business	6 (20.00)	10 (33.33)	16 (26.67)
Mother's Working Status	16 (53.33)	18 (60.00)	34 (56.67)

Experimental: supplemented with quinoa based gluten free biscuits
Control : supplemented with gluten free biscuits without quinoa
Values in parentheses are percentages

Table III: Medical history of celiac children

Medical History	Control (*n*=30)	Experimental (*n*=30)	Total (*n*=60)
Period of suffering from celiac disease			
<6 months	6 (20.00)	8 (26.67)	14 (23.33)
6-12 months	14 (46.67)	4 (13.33)	18 (30.00)
>12 months	10 (33.33)	18 (60.00)	28 (46.67)
Family history of the disease	10 (33.33)	14 (46.67)	24 (40.00)
Relation with subjects*	(*n*=10)	(*n*=14)	(*n*=24)
Mother	6 (20.00)	8 (26.67)	14 (58.33)
Father	8 (26.67)	4 (13.33)	12 (50.00)
Grandparents	6 (20.00)	2 (6.67)	8 (33.33)
Uncle	2 (6.67)	4 (13.33)	6 (25.00)
Aunt	2 (6.67)	2 (6.67)	4 (16.67)
Sickness record			
Morbidity in the last 1 year	14 (46.67)	24 (80.00)	38 (63.33)
Name of Disease*	(*n*=14)	(*n*=24)	(*n*=38)
Fever	12 (85.71)	16 (66.67)	28 (73.68)
Cough and cold	14 (100.00)	12 (50.00)	26 (68.42)
Viral infection	6 (42.85)	2 (8.33)	8 (21.05)
Chicken pox	-	1 (4.17)	1 (2.63)
Dengue	-	1 (4.17)	1 (2.63)

Experimental: supplemented with quinoa based gluten free biscuits
Control : supplemented with gluten free biscuits without quinoa
*Multiple responses, Values in parentheses are percentages
CD- Celiac Disease

Table IV: Prevalence of signs and symptoms among celiac children

Signs and Symptoms*	Control (*n*=30)	Experimental (*n*=30)	Total (*n*=60)
Abdominal bloating/pain	16 (53.33)	26 (86.67)	42 (70.00)
Breathlessness	12 (40.00)	20 (66.67)	32 (53.33)
Chronic diarrhoea	10 (33.33)	18 (60.00)	28 (46.67)
Foul-smelling stools	20 (66.67)	26 (86.67)	46 (76.67)
Blistering skin rash	14 (46.67)	24 (80.00)	38 (63.33)
Fatigue	14 (46.67)	22 (73.33)	36 (60.00)
Canker sores inside mouth	8 (26.67)	16 (53.33)	24 (40.00)
Difficulty in memorizing	10 (33.33)	16 (53.33)	26 (43.33)

Experimental: supplemented with quinoa based gluten free biscuits
Control: supplemented with gluten free biscuits without quinoa
*Multiple responses, Values in parentheses are percentages
CD- Celiac Disease

Table V: Dietary pattern of celiac children

Dietary Pattern	Control (*n*=30)		Experimental (*n*=30)	
Food Habits	Before	After	Before	After
Vegetarian	24 (80.00)	24 (80.00)	18 (60.00)	14 (46.67)
Non-vegetarian	2 (6.67)	2 (6.67)	10 (33.33)	10 (33.33)
Ova- vegetarian	4 (13.33)	4 (13.33)	2 (6.67)	6 (20.00)
School lunch (Tiffin)	28 (93.33)	28 (93.33)	26 (86.67)	30 (100.00)
Preference for fast food	24 (80.00)	24 (80.00)	28 (93.33)	16 (53.33)
Frequency of consuming fast food	(*n*=24)	(*n*=24)	(*n*=28)	(*n*=16)
Once in a fortnight	4 (16.67)	2 (8.33)	8 (28.57)	12 (66.67)
Once in a week	10 (41.67)	8 (33.33)	14 (50.00)	4 (16.67)
Twice in a week	10 (41.67)	14 (58.33)	6 (21.43)	-
Regular in taking meals	22 (73.33)	20 (66.67)	20 (66.67)	28 (93.33)
Frequency of skipping meals				
Breakfast	2 (6.67)	2 (6.67)	6 (20.00)	2 (6.67)
Lunch	6 (20.00)	6 (20.00)	4 (13.33)	-
Type of food consumed				
Homemade	30 (100.00)	30 (100.00)	30 (100.00)	30 (100.00)
Readymade	30 (100.00)	30 (100.00)	30 (100.00)	22 (73.33)
Types of readymade food consumed*				
Haldiram*bhujia*	18 (60.00)	18 (60.00)	20 (66.67)	14 (46.67)
Gluten free bread	12 (40.00)	12 (40.00)	10 (33.33)	14 (46.67)
Gluten free flakes	8 (26.67)	8 (26.67)	12 (40.00)	12 (40.00)
Gluten free *dalia*	2 (6.67)	2 (6.67)	4 (13.33)	4 (13.33)
Gluten free pasta	14 (46.67)	14 (46.67)	12 (40.00)	12 (40.00)
Rice and corn spaghetti	6 (20.00)	6 (20.00)	4 (13.33)	4 (13.33)
Rice cracker	4 (13.33)	4 (13.33)	6 (20.00)	6 (20.00)
Gluten free biscuits/ cookies	30 (100.00)	30 (100.00)	28 (93.33)	30 (100.00)
Others	10 (33.33)	10 (33.33)	14 (46.67)	8 (26.67)

Experimental: supplemented with quinoa based gluten free biscuits
Control: supplemented with gluten free biscuits without quinoa
* Multiple responses, Values in parentheses are percentages
*Bhujia*is an Indian ready to eat snack made from the gram flour.

Table VI: Impact of supplementation on the average intake and percent adequacy of food and nutrient of celiac children

Food and Nutrients	Standard	Control (*n*=30)					Experimental (*n*=30)				
		Before		After		*t*value	Before		After		*t* value
		Av	% A	Av	% A		Av	% A	Av	% A	
Food groups	SDI(g/d)										
Cereals and Millets	180	139.88±26.23	77.71	146.92±17.21	81.62	1.36^{NS}	142.67±24.21	79.26	157.45±18.64	87.47	2.91**
Pulses and Legumes	60	47.05±9.31	78.42	48.18±9.68	80.30	0.44^{NS}	44.85±12.27	74.75	50.95±8.90	84.93	2.23*
GLVs	100	35.02±17.35	35.02	39.23±19.39	39.23	0.94^{NS}	37.05±23.62	37.05	43.67±17.38	43.67	1.47^{NS}
Roots and Tubers	100	69.15±30.20	69.15	73.62±26.93	73.62	0.60^{NS}	54.90±30.10	54.90	78.21±28.78	78.21	4.52**
Other Vegetables	100	48.31±14.78	48.31	43.47±11.58	43.47	1.70^{NS}	43.58±13.57	43.58	47.34±11.79	47.34	1.08^{NS}
Fruits	100	84.81±35.19	84.81	89.28±23.35	89.28	0.53^{NS}	78.02±24.14	78.02	91.01±17.57	91.01	4.09**
Milk and Milk Products (ml)	500	384.47±65.65	76.89	394.67±76.31	78.93	0.57^{NS}	344.54±80.36	68.90	419.25±75.82	83.85	3.31**
Fats andOils	30	33.33±8.46	111.12	36.61±11.88	122.04	1.28^{NS}	31.66±6.93	105.54	35.34±8.94	117.82	1.98^{NS}
Sugars and Jaggery	20	24.18±7.20	120.91	25.92±7.47	129.60	1.28^{NS}	21.69±6.21	108.47	20.99±6.72	104.97	0.34^{NS}
Eggs	50	55.55±3.44	111.1	53.33±3.41	106.66	1.58^{NS}	53.33±1.81	106.66	54.70±1.83	109.4	2.35*
Animal meat	50	45.00±7.07	90.00	40.00±7.05	80.00	1.00^{NS}	40.00±4.20	80.00	42.00±4.21	84.00	1.50^{NS}
Fish	50	35.00±7.07	70.00	30.33±7.04	60.66	0.93^{NS}	30.33±0.00	60.66	30.22±0.17	60.44	1.58^{NS}
Nutrients	EAR										
Energy (Kcal/d)	1700	1571.35±135.9	92.43	1606.81±108.3	94.51	1.22^{NS}	1496.16±125.3	88.00	1619.81±91.04	95.28	4.64**
Carbohydrates (g/d)	100	236.68±35.73	236.68	245.47±23.12	245.47	1.13^{NS}	222.28±18.03	222.28	246.89±28.82	246.89	3.96**
Protein (g/d)	19	24.66±2.85	129.78	26.51±4.10	139.52	1.81^{NS}	26.32±3.34	138.52	29.93±3.18	157.52	4.00**
Visible Fat (g/d)	30	33.33±8.61	111.1	36.61±12.09	122.03	1.28^{NS}	31.66±7.05	105.53	35.34±9.10	117.8	1.98^{NS}
Iron (mg/d)	10	12.26±1.96	122.6	11.64±2.17	116.4	1.84^{NS}	10.06±2.20	100.6	13.12±1.82	131.2	5.10**
Calcium (mg/d)	500	461.03±58.11	92.20	470.51±51.03	94.10	1.71^{NS}	437.80±56.66	87.56	480.36±59.36	96.07	2.63**
Thiamine (mg/d)	1.0	0.65±0.11	65.00	0.67±0.12	67.00	0.66^{NS}	0.63±0.12	63.00	0.68±0.09	68.00	2.04*
Riboflavin (mg/d)	1.3	0.73±0.11	56.15	0.77±0.09	59.23	1.62^{NS}	0.74±0.15	56.92	0.78±0.12	60.00	1.26^{NS}
Niacin (mg/d)	10	8.81±1.76	88.1	8.99±1.69	89.9	0.48^{NS}	8.43±1.73	84.3	9.01±1.35	90.1	1.63^{NS}
Folic acid (µg/d)	142	96.1±9.35	67.67	93.91±7.44	66.13	0.99^{NS}	92.14±16.07	64.88	99.41±14.42	70.00	1.95^{NS}
β-carotene (µg/d)	$4800^{\#}$	2478.87±911.9	51.64	2538.57±833.9	52.88	0.34^{NS}	2334.08±1017	48.62	2593.10±835.3	54.02	1.12^{NS}
Vitamin C (mg/d)	36	25.66±5.14	71.27	24.82±3.93	68.94	0.91^{NS}	23.93±4.93	66.47	26.42±4.87	73.38	1.93^{NS}

Experimental: supplemented with quinoa based gluten free biscuits

Control: supplemented with gluten free biscuits without quinoa

Values are Mean±*SD*

AV: Average daily food or nutrient intake, % A: Percent adequacy of daily food or nutrient intake

*significant at 5% level of significance, **significant at 1% level of significance, NS- Non-significant
Suggested dietary intake (ICMR 2010),Estimated average requirement (ICMR 2020), # Recommended dietary allowance (ICMR 2010)

Table VII: Impact of supplementation on the anthropometric profile of celiac children

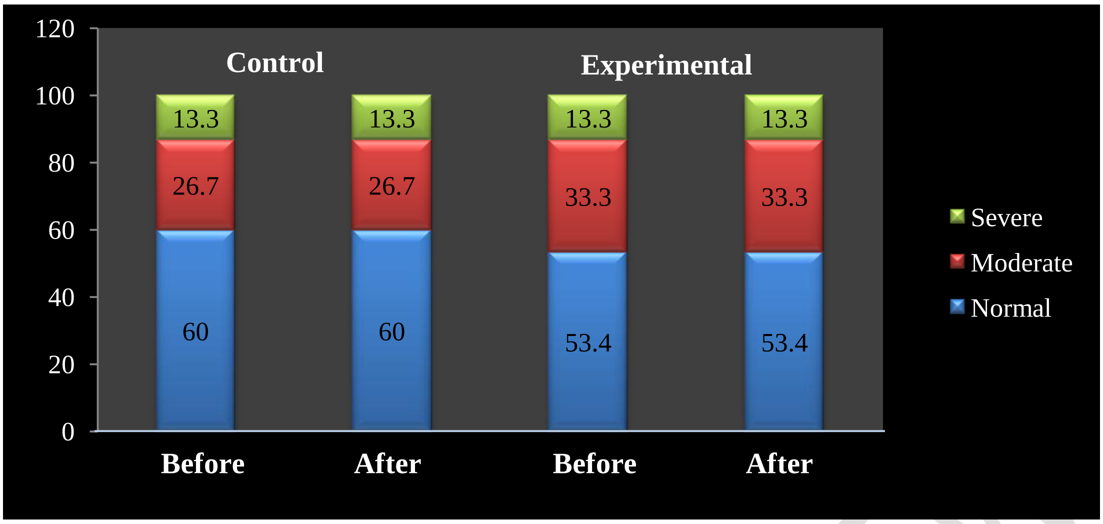
Anthropometric Measurements	Control (*n*=30)				Experimental (*n*=30)			
	Before	After	% change	*t* value	Before	After	% change	*t* value
Height (cm)	127.22±7.49	127.42±7.68	0.15	0.09^{NS}	127.02±9.01	127.12±9.00	0.08	0.04^{NS}
Weight (kg)	20.84±3.03	21.02±2.87	0.86	1.87^{NS}	21.86±3.27	23.36±3.23	6.86	14.49**
BMI (Kg/m^2)	12.84±1.20	12.92±1.15	0.65	0.27^{NS}	13.57±1.81	14.48±1.70	6.70	2.003*
MUAC (cm)	20.94±2.35	20.98±2.38	0.16	1.16^{NS}	19.84±2.16	19.90±2.15	0.27	4.00**

Experimental: supplemented with quinoa based gluten free biscuits
Control: supplemented with gluten free biscuits without quinoa
Values are Mean±*SD*
BMI: Body Mass Index, MUAC: Mid UpperArm Circumference
*Significant at 5% level of significance
**Significant at 1% level of significance
NS-Non-significant

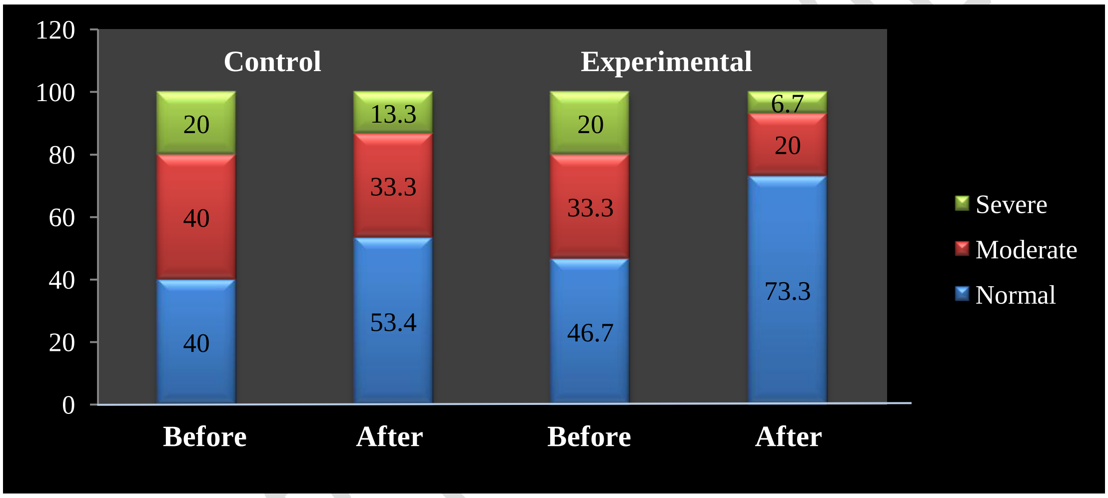
Table VIII:Impact of supplementation on the biochemical parameters of celiac children

Parameters	Control (*n*=30)			Experimental (*n*=30)			Normal range
	Before	After	*t*value	Before	After	value	
Hb (gm/dl)	12.04±0.71	12.11±0.73	1.17^{NS}	11.59±0.75	12.1±0.53	3.75**	12.0-17.0
PCV (%)	44.07±4.17	44.21±4.18	0.53^{NS}	46.67±2.28	47.06±2.47	0.83^{NS}	45.0-55.0
RBC count (10^6/ul)	4.52±0.18	4.55±0.27	0.50^{NS}	4.60±0.28	4.66±0.28	0.71^{NS}	4.5-5.5
MCV (fl)	87.94±4.47	88.10±5.92	0.18^{NS}	85.28±4.09	85.49±3.21	0.23 ^{NS}	83.0-101.0
Anti-tTG IgA (U/ml)	93.61±50.76	71.96±39.00	1.94^{NS}	116.88±60.08	57.19±24.18	6.46**	<7

Experimental: supplemented with quinoa based gluten free biscuits
Control: supplemented with gluten free biscuits without quinoa
Values are Mean±*SD*
Hb: Haemoglobin, PCV: Packed Cell Volume, RBC: Red Blood Count,
MCV: Mean Corpuscular Volume, tTG: tissue Transglutaminase, IgA: Immunoglobulin A
**significant at 1% level of significance
NS- Non-significant



Height-for-age



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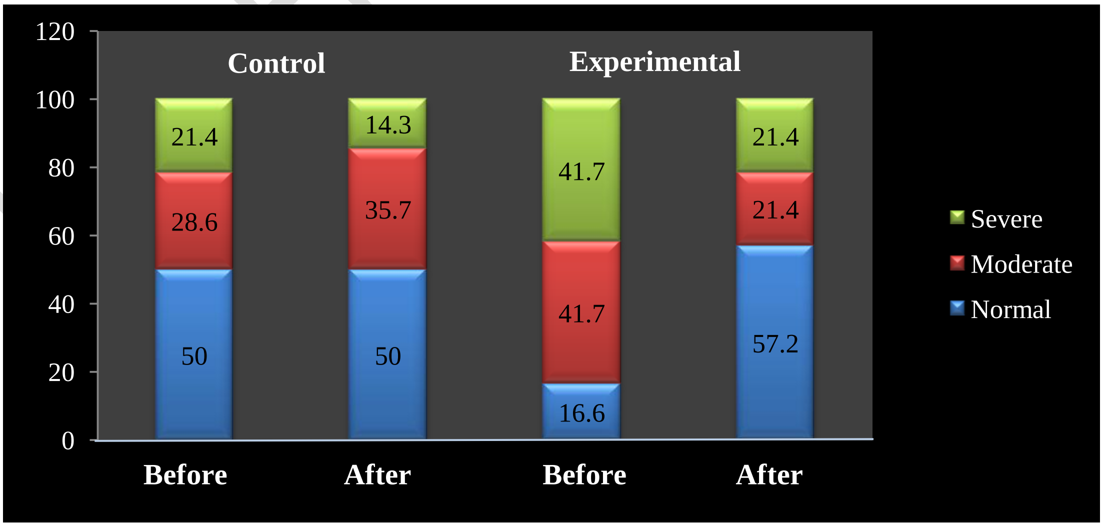

BMI-for-age

FIGURE I: Impact of supplementation on prevalence of malnutrition in celiac children